REMARKS/ARGUMENTS

Claims 1, 4-11, 13-14, 17-24, and 38-39 are pending.

Claims 1, 4-5, 7, 10, 13-14, and 17-24 have been amended.

Claims 1 and 4-11 have been withdrawn.

Claims 2-3, 12, 15-16, and 25-37 have been cancelled.

Support for the amendments is found in the claims and specification, as originally filed. Specifically, support for claim 1 can be found on pages 8-10, 16-17, 20-21, 23, 25, 35, lines 4-8, the Examples, and claims 2-3. Support for claim 13 can be found on pages 11-13; 31-32, 21, lines 10-12, the Examples, and claims 15-16. Support for the limitation "a kit" and claim 39 can be found on page 31, line 9, to page 14, line 3, and the Examples. Support for claim 38 can be found on pages 38-63 (the Examples).

No new matter is believed to have been added.

Applicants wish to thank the Examiner and her Supervisor for a meeting on August 19, 2008. We explained to the Examiners what components are included in the claimed reagent for selective measuring triglycerides ("TG") and how TG are selectively measured by using the method of claim 1. The Examiner pointed that the claimed "reagent" is actually "a kit" because it comprises at least two separate containers which also may comprise sub-containers with different reagents. We explained to the Examiners that the inventors have found that a combination of two types of lipases, specific reaction promoters, and a specific m/n ratio of the added first and second reaction promoters allows *selectivity* of the TG measurements. We also discussed the proposed amendments to overcome the obviousness rejection. The Examiners indicated willingness to reconsider the obviousness rejection.

The claims are rejected under 35 U.S.C. 103(a) over Okada et al., WO00/60112, Miyauchi et al., EP 1148142, and Matsui et al., US 6,194,164.

The rejection is traversed because the combination of the references does not describe or suggest a kit and a method for selective measurement of triglycerides contained in very low density lipoprotein and intermediate density lipoprotein comprising:

(a) a first lipoprotein lipase contained in the first reagent that depends on the concentration of a surfactant, and a second lipoprotein lipase contained in the second reagent that hardly depends on the concentration of a surfactant; and

(b) two different selective reaction promoters that satisfy the following condition: an m/n ratio is in the range of 1.1 to 1.2, where m is the average mole number of the added polyoxyalkylene in its ether or ester compound which is used as the first selective reaction promoter and n is the average mole number of the added polyoxyalkylene in its ether or ester compound which is used as the second selective reaction promoter.

The claimed kit and method allow an easy and accurate selective measurement of triglycerides ("TG") contained in VLDL and IDL (page 3 of the present specification). Since the triglycerides contained in the low density lipoprotein and the high density lipoprotein have been already eliminated in the first step, only the triglycerides contained in the very low density lipoprotein and intermediate density lipoprotein or in the very low density lipoprotein react with lipoprotein lipase to generate glycerol in the second step (see pages 8-10). When adding catalase to the first reagent as an enzyme that catalyzes the reaction leading to the conversion of hydrogen peroxide unto another substance, a substance that inhibits the activity of the catalyse (e.g., sodium azide) is added to the second reagent so that the hydrogen peroxide generated in the second step is not eliminated by the catalyse. However, when adding peroxidase to the first reagent as an enzyme that catalyzes the reaction leading to the conversion of hydrogen peroxide into another substance, a substance that inhibits the activity of the enzyme is not added to the first reagent (see page 35, lines 4-11, and pages 20-22).

Also, the first and a second reagent promoters are different in at least an average mole number of the added polyxyalkylene because the ratio of **m/n** is from 1.1 to 1.2 (i.e., 1 is not within the range), where **m** is the average mole number of the added polyoxyalkylene in its ether or ester compound which is used as the first selective reaction promoter and **n** is the average mole number of the added polyoxyalkylene in its ether or ester compound which is used as the second selective reaction promoter. *See* Table 2 and the description on page 42, line 19, through page 46, line 4.

Okada et al. describe reagents for measuring triglycerides in a sample comprising two reagents. A first reagent comprises glycerol kinase, glycerol-3-phosphate oxidase, and catalase, while a second reagent comprises a lipoprotein lipase (see page 35, working example 1 of the automated English translation).

Miyauchi et al. describe a method of quantifying TG in HDL and LDL (see [0025]-[0030] and [0038]-[0048] and the Examples) using only one lipoprotein lipase. For measuring TG in HDL, reagents for inhibiting the reaction of lipoproteins other than HDL (e.g., aggregating agents) are added to prevent decomposition of TG in LDL and VLDL by a lipoprotein lipase and then the free glycerol contained in the sample is eliminated ([0026]-[0029]). Subsequently, a lipoprotein lipase and other enzymes are added to generate hydrogen peroxide from HDL [0029]. A similar process is used for quantifying TG in LDL.

Matsui et al. describe a reagent for measuring cholesterol in LDL comprising the cholesterol esterase and cholesterol oxidase in the first reagent (col. 2, lines 59-62; tables 1-2 and 4). Matsui et al. do not describe using a lipoprotein lipase.

Further, Matsui et al. describe using specific surfactants having specific different hydrophilic-lipophilic balance ("HLB") in the first and second measurement steps, e.g., HLB is 13-15 in the first step, and 11-13 in the second step (see col. 3-4).

Matsui et al. do not teach adjusting HLB values so as surfactants act on different lipoproteins, e.g., IDL and VLDL, and different lipoprotein lipases, not to mention, adjusting a ratio of the average amount of moles of polyoxyalkylene in its ether or ester compound.

The HLB value reflects a degree to which a surfactant is hydrophilic or lipophilic (see the enclosed pages from "Wikipedia", accessed at http://en.wikipedia.org on 12/5/2007, 2 pages). The HBL value does not show how many moles of a surfactant is added and a ratio of the surfactants in two steps.

Thus, Okada et al., Miyauchi et al., and Matsui et al. do not describe or suggest using two different lipases having different activities in two different reagents and selecting a specific molar ration of the two selective reagents.

As described in the present specification, the claimed kit provide high selectivity to VLDL and IDL (e.g., pages 43-46). When the average mole number ration is 1.1.-1.2, the triglycerides content in VLDL and IDL can be selectively measured, compared to those contained in chylomicron, LDL, and HDL (page 44, third full paragraph).

The Examiner is of the opinion that it would have been obvious to modify the reagent of Okada et al. and select a particular mole number ratio of the polyoxyalkylene derivatives based upon the teaching of the calculation of HLB values and art-recognized method of using surfactants with the particular HLB values to stabilize specific lipoproteins (pages 5-6 of the Official Action).

However, the methods and reagents of Okada et al., Miyauchi et al., and Matsui et al. are different from each other because they use different enzymes, measure different substance (triglycerides or cholesterol), and exhibit different behaviors towards surfactants such as polyalkylene oxide derivatives.

For example, Okada et al. describes that BL-9EX (POE(9) lauryl ether) which is a polyalkylene oxide derivative can make an enzyme such as lipoprotein lipase selectively react

with triglycerides in LDL and HDL (see page 39, eighth paragraph, of the automated English translation). BL-9EX has HLB value 14.5 (see Annex 1). Matsui et al. describe that polyalkylene oxide derivatives having HLB values of not less than 13 and not more than 15 can make cholesterol esterase and cholesterol oxidase selectively react with cholesterol in lipoproteins other than LDL (i.e., HDL, VLDL, CM and the like; col. 3, lines 17-37).

Further, Okada et al. describe that KF-351 (polyether-modified silicone oil) which is polyalkylene oxide derivative can make an enzyme such as lipoprotein lipase selectively react with triglycerides in IDL and HDL (see page 39, seventh paragraph, of the automated English translation). KF-351 has HLB value of 12 (see Annex 2 and 3). Matsui et al. describe that polyalkylene oxide derivatives having HLB values of not less than 11 and not more than 13 can make a cholesterol esterase and cholesterol oxidase selectively react with cholesterol in all lipoproteins (i.e., HDL, VLDL, CM, and LDL; col. 4, lines 12-39).

Thus, it would not have been obvious what surfactants and HLB values to select based on the contradictory data of Okada et al. and Matsui et al. for the selective measurement of TG in VLDL and IDL.

Thus, Okada et al., Miyauchi et al., and Matsui do not make the claimed kit and method obvious. Applicants request that the rejection be withdrawn.

A Notice of Allowance for all pending claims is requested.

Respectfully submitted,

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